

Ovarian Cancer: Loss of Heterozygosity Frequently Occurs in the ATM Gene, but Structural Alterations Do Not Occur in This Gene

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Key Words

ATM gene · Chromosome 11q23 · Ovarian cancer

Abstract

Ataxia-telangiectasia is a multisystem recessive disease characterized clinically by cerebellar ataxia, oculocutaneous telangiectasias, immunodeficiency, sensitivity to radiomimetic agents and an increased predisposition to cancer. This pleiotropic disorder is caused by mutations in the ATM gene, which is located at the human chromosomal region 11q23. Loss of heterozygosity (LOH) at 11q22-q23 is a frequent event in ovarian cancer, suggesting the presence of a tumor suppressor gene in this region. We have found that LOH in the ATM gene occurred in 44% of informative cases in a series of 22 primary ovarian tumors. LOH of this region occurred at the same frequency during the advanced stages (III-IV; 3/9, 33%) as in the early stages (I-II; 4/13, 31%) of ovarian cancer. To investigate the role of ATM in ovarian cancer, we used a PCR-based single-strand conformation polymorphism assay for mutation detection of the entire coding sequence of the ATM gene (65 exons) in 22 ovarian tumors. No somatic alterations of the ATM gene were found in these ovarian cancer samples including those

with LOH present in the ATM gene. Our study has identified a region (11q23) which probably contains a frequently altered tumor suppressor gene in ovarian cancer, and this gene does not appear to involve the coding sequences of the ATM gene.

Introduction

Genetic linkage studies mapped the ataxia-telangiectasia (AT) locus to chromosomal region 11q22-23 [1], a region that frequently exhibits loss of heterozygosity (LOH) in several types of tumors [2-8]. Using a positional cloning approach, the ATM gene (mutated in AT) has recently been identified [9, 10]. The gene is expressed as a 12-kb transcript in all tissues and cell types [9].

Individuals carrying two mutant ATM alleles and affected by AT have a 100-fold higher risk of developing cancer than unaffected age-matched subjects [11]. The cancers most frequently identified in this group are leukemias and lymphomas. A higher incidence of tumors has also been observed in obligate AT heterozygote carriers with the relative risk for females and males being 3.5 and 3.8, respectively [11]. The strongest correlation between

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Exhibit 4

development of cancer in AT carriers was found for breast cancer. Recent studies have shown a relative risk of about 5 in one study [11] and 3.9 in another more recent study that involved 58 cases of breast cancer [12]. However, no somatic alterations of the ATM gene were found in 38 unselected breast tumor samples [13]. An excess risk of ovarian cancer may also occur in heterozygotes for AT [14]. LOH on chromosome arm 11q was identified in 67% of ovarian carcinomas, which was highly suggestive of the presence of an important tumor suppressor gene (TSG) on this chromosomal arm [2]. Studies using micro-cell-mediated chromosome transfer into carcinoma cell lines have also shown that the long arm of chromosome 11 is able to suppress tumorigenesis [15].

To investigate the role of ATM in ovarian cancer, we examined LOH in the ATM gene and used an exon-scanning polymerase-chain-reaction (PCR)-based single-strand conformation polymorphism (PCR-SSCP) assay in order to examine DNA from 22 ovarian tumors for ATM mutations. We found that LOH frequently occurred (44%) at the ATM gene in ovarian cancer. However, no structural alterations of the ATM gene were found, suggesting that an important TSG is in the region of the ATM gene and it is probably altered in ovarian cancer.

Materials and Methods

Samples and Staging of Ovarian Cancer

Twenty-two samples of DNA from primary ovarian cancers were fresh tissues obtained at the time of surgery after informed consent was obtained from each patient. Adjacent noncancerous tissues or peripheral blood lymphocytes were also obtained from each individual. The tumors were histologically classified according to the Histological Typing of Ovarian Tumors by the World Health Organization and staged according to the International Federation of Gynecology and Obstetrics staging system; each of these samples contained more than 80% tumor cells. These cancers included 7 clear-cell adenocarcinomas, 6 serous adenocarcinomas, 3 mucinous cystadenocarcinomas, 2 mesodermal mixed tumors, 2 mixed adenocarcinomas and 2 endometrial adenocarcinomas. High-molecular-weight DNA was prepared by proteinase K digestion and phenol/chloroform extraction as described [16].

Analysis of LOH with Microsatellite Markers

The LOH analysis was performed by PCR amplification of microsatellite sequences [17]. The following microsatellite loci were used at 11q22-q23: D11S2000, D11S2179, D11S1818, D11S897 [18]. The PCR reaction contained 10–100 ng of DNA, 10 pmol of the primers, 2 nmol of the four deoxyribonucleotide triphosphates (Pharmacia, Stockholm, Sweden), 0.5 unit of Taq DNA polymerase (Boehringer-Mannheim, Indianapolis, Ind., USA) and 2 μ Ci of 32 P- α -dCT (ICN, Irvine, Calif., USA) in 20 μ l of the specified buffer with 1.5 mM MgCl₂. Samples were amplified by 35 cycles of denaturing

for 36 s at 94°C, annealing for 33 s at 55°C and extension for 1 min at 72°C in a Programmable Thermal Controller (MJ Research Inc., Watertown, Mass., USA). After amplification, PCR samples were diluted 5-fold in loading buffer containing 20 mM EDTA, 94% formamide and 0.05% of both bromophenol blue and xylene cyanol. The products were heated to 95°C for 5 min, and 15 μ l of each sample was electrophoresed through a 5–6% polyacrylamide gel containing 8.3 M urea for 3–4 h at 85 W. Subsequently, the gels were dried and subjected to autoradiography on Kodak XAR film at –80°C.

Relative intensities of DNA fragments in normal and tumor lanes were examined with a Fujix Bas 2000 System. In this examination, allelic loss was scored to have occurred when the intensity of one allelic fragment in the tumor was less than 40% of that in the corresponding normal tissue.

The PCR amplification of the entire coding region of ATM was examined as previously reported by the incorporation of 32 P- α -dCTP, using the primers from the genomic sequences and the optimal annealing temperatures for each of the exons as previously reported (total number of exons is 65) [19]. The products were separated in 0.5 \times HydroLink MDE Gel (J.T. Baker Inc., Phillipsburg, N.J., USA) at room temperature. The gels were dried and exposed to X-ray films at –80°C overnight.

Results

Overall, 7 of 22 (32%) cases had LOH in at least one locus (D11S2000, D11S2179, D11S897, D11S1818) either flanking or in the ATM gene. LOH at D11S2179, a marker located within the ATM locus, occurred in 7 of 16 (44%) informative cases (fig. 1). Correlation of these results with the clinicopathological characteristics of the patients and their tumors showed that LOH in this region (11q23) occurred at the same frequency in advanced stages (III–IV; 3/9, 33%) as in early stages (I–II; 4/13, 31%) of ovarian cancer (table 1).

The 65 exons of the entire ATM gene from DNA of each of the 22 ovarian cancer samples were examined by PCR-SSCP (fig. 2a). No alterations were found (fig. 2b).

Discussion

Our study confirms the high frequency of LOH in primary ovarian cancer at 11q23 [20, 21]. Furthermore, we found a hotspot of LOH within the ATM gene (44%) at 11q23. However, the ATM gene does not appear to be the TSG that is altered in these sporadic ovarian carcinomas, because we could not find structural alterations of this gene. Using a similar SSCP technique, we were able to detect frequent mutations in the p53 gene in lung tumors [22]; therefore, we believe that it is unlikely that we have a very high rate of false-negative results in the present sam-

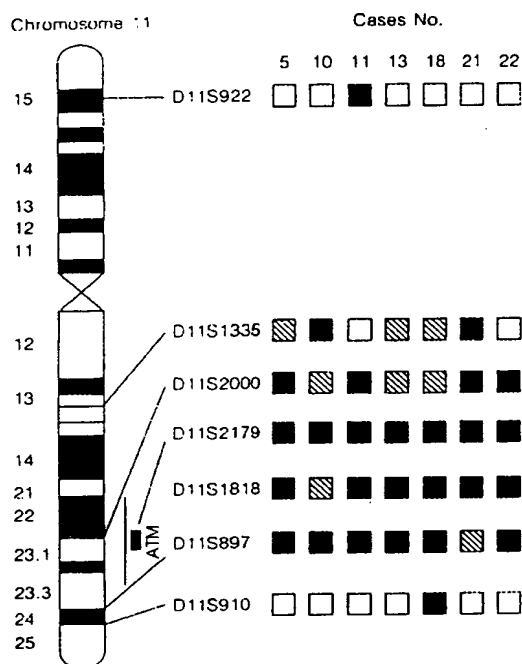


Table 1. Clinical characteristics of ovarian cancer patients

Case No.	Stage	Histologic type	LOH (11q23)
1	Ia	CCA	+
2	Ia	CCA	+
3	Ia	SA	+
4	Ic	CCA	+
5	Ic	CCA	LOH
6	Ic	CCA	+
7	Ic	MCA	+
8	Ic	SA	+
9	Ic	MMT	+
10	IIc	CCA	LOH
11	IIc	MA	LOH
12	IIc	MCA	+
13	IIc	MMT	LOH
14	IIIa	CCA	+
15	IIIb	EA	+
16	IIIb	MA	+
17	IIIc	MCA	+
18	IIIc	SA	LOH
19	IIIc	SA	+
20	IV	EA	+
21	IV	SA	LOH
22	IV	SA	LOH

CCA = Clear-cell adenocarcinoma; SA = serous adenocarcinoma; MCA = mucinous cystadenocarcinoma; MMT = mesodermal mixed tumor; MA = mixed adenocarcinoma; EA = endometrial adenocarcinoma; + = retention of heterozygosity.

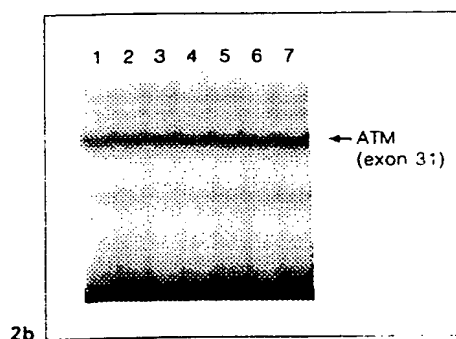
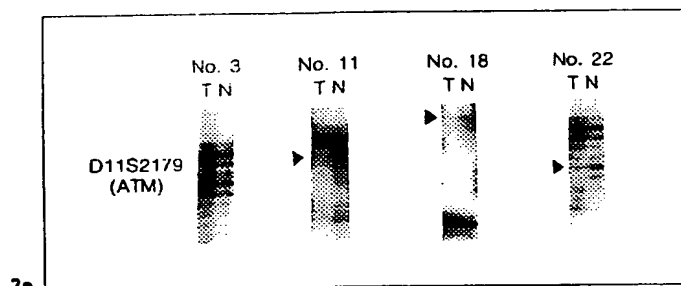


Fig. 1. Summary of LOH analysis of chromosome 11q23 in ovarian cancer. Seven samples which showed LOH at one or more loci are presented. For the markers which are assigned to the same location by linkage analysis, the results obtained from each marker were combined. The status of each chromosomal locus is indicated by shading as LOH (black), retention of heterozygosity (white) and noninformative (hatched). Patient numbers are listed at the top of each column. **Fig. 2.** **a** Representative autoradiographs showing LOH. Sample No. 3 retains heterozygosity in the ATM gene. Samples No. 11, 18 and 22 show LOH at the ATM gene. Arrowheads point to alleles showing loss. T = Tumor DNA; N = normal DNA. **b** SSCP analysis of ATM gene exon 31 in primary ovarian samples. No alterations were found. Lane 1 = Sample No. 5; lane 2 = sample No. 10; lane 3 = sample No. 11; lane 4 = sample No. 13; lane 5 = sample No. 18; lane 6 = sample No. 21; lane 7 = sample No. 22.

ples. Furthermore, the average size of genomic fragments examined by SSCP was approximately 240 bp which is well below the size limitation generally believed to be necessary to detect more than 80% of the mutations [23]. We cannot exclude the possibility that alterations affecting function of the ATM gene occurred outside the coding region of the gene.

We found that LOH at 11q23 occurred at the same frequency in advanced stages (III-IV; 3/9, 33%) and early stages (I-II; 4/13, 34%) of the disease (table 1). These results suggest that LOH at 11q23 occurs relatively early in progression of ovarian cancer.

Using an effective and rapid DNA screening method to identify ATM mutations, we detected no somatic alter-

ations of the ATM gene in 22 ovarian tumor samples, including those with LOH in the ATM gene at 11q23. Although a larger data set will be required to reveal the exact frequency of AT carriers in ovarian cancer patients, our data suggest that the ATM gene is infrequently altered in ovarian cancer, and another TSG, which is important in ovarian cancer, is present in the region of 11q23.

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References

- Gatti RA, Berkel I, Boder E, Braedt G, Charmley P, Concannon P, Ersoy F, Foroud T, Jaspers NG, Lange K, Lathrop GM, Leppert M, Nakamura YY, O'Connell P, Paterson M, Salsner W, Ozden S, Silver J, Sparkes RS, Susi E, Weeks DE, Weis S, White R, Yoder F: Localization of an ataxia-telangiectasia gene to chromosome 11q22-q23. *Nature (Lond)* 1988;336:577-580.
- Foulkes WD, Campbell IG, Stamp GWH, Trowsdale J: Loss of heterozygosity and amplification on chromosome 11q in human ovarian cancer. *Br J Cancer* 1993;67:268-273.
- Gustafson CE, Young J, Leggett B, Searle J, Chenevix-Trench G: Loss of heterozygosity on the long arm of chromosome 11 in colorectal tumors. *Br J Cancer* 1994;70:395-397.
- Bethwaite PB, Koreth J, Herrington CS, McGee JO: Loss of heterozygosity occurs at the D11S29 locus on chromosome 11q23 in invasive cervical carcinoma. *Br J Cancer* 1995;71:814-818.
- Hampton GM, Penny LA, Baergen RN, Larson A, Brewer C, Liao S, Busby-Earle RMC, Williams AWR, Steel CM, Bird CC, Stanbridge EJ, Evans GA: Loss of heterozygosity in cervical carcinoma: Subchromosomal localization of a putative tumor-suppressor gene to chromosome 11q22-24. *Proc Natl Acad Sci USA* 1994;91:6953-6957.
- Negrini M, Rasio D, Hampton GM, Sabbioni S, Rattan S, Carter SL, Rosenberg AL, Schwartz GF, Shiloh Y, Cavence WK, Croce CM: Definition and refinement of chromosome 11 regions of loss of heterozygosity in breast cancer: Identification of a new region at 11q23.3. *Cancer Res* 1995;55:3003-3007.
- Rasio D, Negrini M, Manenti G, Dragani TA, Croce CM: Loss of heterozygosity at chromosome 11q in lung adenocarcinoma: Identification of three independent regions. *Cancer Res* 1995;55:3988-3991.
- Herbst RA, Larson A, Weiss J, Cavence WK, Hampton GM, Arden KC: A defined region of loss of heterozygosity at 11q23 in cutaneous malignant melanoma. *Cancer Res* 1995;55:2494-2496.
- Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, Tagle DA, Smith S, Uziel T, Sfez S, Ashkenazi M, Pecker I, Frydman M, Harnik R, Patanjali SR, Simmons A, Clines GA, Sartiell A, Gatti RA, Chessa L, Sanal O, Lavin MF, Jaspers NGJ, Taylor AMR, Arlett CF, Miki T, Weissman SM, Lavett M, Collins FS, Shiloh Y: Single ataxia telangiectasia gene with a product similar to PI-3 kinase. *Science* 1995;268:1749-1753.
- Savitsky K, Sfez S, Tagle D, Ziv Y, Sartiell A, Collins FS, Shiloh Y, Rotman G: The complete sequence of the coding region of the ATM gene reveals similarity to cell cycle regulators in different species. *Hum Genet* 1995;4:2025-2032.
- Swift M, Reitnauer PT, Morrell D, Chase CL: Breast and other cancers in families with ataxia-telangiectasia. *N Engl J Med* 1987;316:1289-1294.
- Easton DF: Cancer risk in A-T heterozygotes. *Int J Radiat Biol* 1994;66:177-182.
- Vorechovsky I, Rasio D, Luo L, Monaco C, Hammarstrom L, Webster ADB, Zaloudik J, Barbanti-Brodano G, James M, Russo G, Croce CM, Negrini M: The ATM gene and susceptibility to breast cancer: Analysis of 38 breast tumors reveals no evidence for mutation. *Cancer Res* 1996;56:2726-2732.
- Swift M, Sholman L, Perry M, Chase C: Malignant neoplasms in the families of patients with ataxia-telangiectasia. *Cancer Res* 1976;36:209-215.
- Negrini M, Sabbioni S, Pessari L, Rattan S, Corallini A, Barbanti-Brodano G, Croce CM: Suppression of tumorigenicity of breast cancer cell by microcell mediated chromosome transfer: Studies on chromosomes 6 and 11. *Cancer Res* 1994;54:1331-1336.
- Takano H, Okamoto A, Terashima Y, Yokota J: High incidence of allelic loss at the RB gene locus in advanced human ovarian cancer. *Int J Oncol* 1994;6:129-135.
- Koike M, Takeuchi S, Yokota J, Park S, Hatta Y, Miller CW, Tsuruoka N, Koeffler HP: Frequent loss of heterozygosity in the region of the D7S523 locus in advanced ovarian cancer. *Genes Chromosome Cancer* 1997;19:1-5.
- Vanagaite L, James MR, Rotman G, Savitsky K, Bar-Shira A, Gilad S, Ziv Y, Uchenik V, Sartiell Y, Collins FS, Sheffield VC, Richard CW III, Weissenbach J, Shiloh Y: A high-density microsatellite map of the ataxia-telangiectasia locus. *Hum Genet* 1995;95:451-454.
- Shiohara M, El-Deiry WS, Wada M, Nakamaki T, Takeuchi S, Yang G, Chen D-L, Vogelstein B, Koeffler HP: Absence of WAF1 mutations in a variety of human malignancies. *Blood* 1994;84:3781-3784.
- Davis M, Hitchcock A, Foulkes WD, Campbell IG: Refinement of two chromosome 11q regions of loss of heterozygosity in ovarian cancer. *Cancer Res* 1996;56:741-744.
- Gabra H, Watson EV, Taylor KJ, Mackay J, Leonard RCF, Steel CM, Porteous DJ, Smyth JF: Definition and refinement of a region of loss of heterozygosity at 11q23.3-q24.3 in epithelial ovarian cancer associated with poor prognosis. *Cancer Res* 1996;56:950-954.
- Hayashi K: PCR SSCP: Single-strand conformation polymorphism analysis of PCR products. In: Londegren U (ed): *Laboratory Protocols for Mutation Detection*. Oxford, Oxford University Press, 1996, pp 14-22.
- Miller CW, Aslo K, Yokota J, Koeffler HP: p53 mutation in human lung tumors. *Cancer Res* 1992;52:1695-1699.